Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Mucosal homeostasis is altered in the ileum of gnotobiotic mice

Chasen J. Greig, MD,[a] *Arik Alper, MD,*[b] *Andrew L. Goodman, PhD,*[c] *and Robert A. Cowles, MD*[a,*]

[a] *Section of Pediatric Surgery, Department of Surgery, Yale School of Medicine, New Haven, Connecticut*
[b] *Section of Pediatric Gastroenterology, Department of Pediatrics, Yale School of Medicine, New Haven, Connecticut*
[c] *Department of Microbial Pathogenesis, Yale School of Medicine, New Haven, Connecticut*

ARTICLE INFO

Article history:
Received 23 February 2018
Received in revised form
20 May 2018
Accepted 24 May 2018
Available online xxx

Keywords:
Microbiome
Gnotobiotic
Mucosal homeostasis
Intestine
Mouse

ABSTRACT

Background: The microbiome of the gastrointestinal tract is a vast collection of microorganisms implicated in numerous aspects of normal physiology and disease pathogenesis. The use of gnotobiotic mouse models, with single or specific communities of microbes comprising the microbiome, can enhance our understanding of the microbiome-host relationship. We hypothesized that gnotobiotic mice would exhibit differences in mucosal homeostasis when compared with mice with conventional flora (CF).

Materials and methods: Single-organism gnotobiotic mice were generated containing *Escherichia coli* MG1655, *Akkermansia muciniphila*, *Bacteroides eggerthii*, and *Clostridium symbiosum*, representing four of the major phyla present in the gastrointestinal tract. Distal ileal segments were harvested from adult mice, and histologic sections were H&E stained and used to measure villus height and crypt depth. Immunohistochemistry was performed with Ki67 and TUNEL as markers of proliferation and apoptosis, respectively.

Results: When compared to the ileum from CF mice, the ileum from all groups of gnotobiotic mice had significant increases in nearly all measured parameters. In addition, significant differences were seen among certain gnotobiotic groups for villus height, crypt depth, and apoptosis.

Conclusions: Single-organism gnotobiotic mice demonstrate enhanced morphometric parameters compared with mice with CF and show differences in growth patterns among bacterial species. These findings suggest unique interactions between individual bacteria and the host animal which hold potential for future therapeutic strategies aimed at mucosal restoration. The mechanisms involved in this process therefore warrant further study.

Introduction

The intestinal microbiome is a complex mixture of nonpathogenic bacteria that lie in close association with host mucosal surfaces of the gastrointestinal tract.[1] The role of the microbiome in human disease has been a popular area of study in recent years, with investigators uncovering critical contributions to the host immune system, nutrition, metabolism, and gastrointestinal disease.[2] Much of the progress in this field has been made possible through the use of germ-free mice, which lack exposure to any microorganisms, and gnotobiotic mice, which have been exposed to a single or specific collection of

* *Corresponding author.* Section of Pediatric Surgery, Department of Surgery, Yale School of Medicine, 330 Cedar Street, FMB 131, New Haven, CT 06510. Tel.: +1 203 785 2701; fax: +1 203 785 3820.
E-mail address: robert.cowles@yale.edu (R.A. Cowles).
0022-4804/$ – see front matter
https://doi.org/10.1016/j.jss.2018.05.055

microorganisms.[3,4] Although the focus has been primarily on the role of the microbiome in host immunology and disease, its role in intestinal development and homeostasis is beginning to be delineated.[5-7] These data suggest a potential role of the microbiome in intestinal adaptation and thus in the management of short bowel syndrome (SBS). Although intestinal adaptation encompasses a significant number of morphologic and functional changes, anatomic changes such as villus height (VH) and crypt depth (CD) are generally used to assess for the presence of adaptation. In the present study, we use mucosal growth as determined by the morphometric parameters VH and CD and intestinal crypt cell proliferation as markers of intestinal mucosal homeostasis. We hypothesized that gnotobiotic mice would have differences in intestinal morphometric and proliferative parameters compared with mice with conventional flora (CF). We feel this would provide evidence supporting the concept of targeting the unique interactions of certain microbes with the host epithelium such that mucosal growth could be stimulated.

Materials and methods

Animals

Adult male wild-type C57Bl/6 mice housed under standard conditions (12 h light/dark cycle, standard chow/water *ad libitum*) at the Yale Animal Resources Center were deemed to harbor CF. Gnotobiotic mice were generated at the Microbial Sciences Institute at Yale under the following conditions: adult germ-free mice were orally gavaged with distinct single strains of bacteria such as *C symbiosum*, *B eggerthii*, *A muciniphila*, and *E coli* MG1655. Level of colonization was determined via serial dilution plating of fresh fecal samples in an anaerobic chamber. Gnotobiotic mice were kept in four different isolators to avoid cross contamination and received identical diets consisting of sterile chow/water *ad libitum*. All mice were sacrificed once a steady level of bacterial viability was achieved, with all animals in the study sacrificed between 8 and 10 wk of age. For anatomic measurements, each gnotobiotic group consisted of three animals, whereas the CF group consisted of four animals. All animal protocols were approved by the Yale University's Institutional Animal Care and Use Committee.

Tissue procurement and preparation

After CO_2 asphyxiation of the mice, an abdominal incision was used to deliver the intestines. The ileocecal junction was identified, and the ileum was transected at this point. Beginning with the distal end, the mesentery was cleared proximally to the ligament of Treitz, and the proximal jejunum was transected at this point. The bowel was submerged in ice-cold PBS, and catheter syringe was used to flush with PBS to remove fecal material. Two-centimeter distal ileal segments were obtained from predetermined areas measured from the distal end. Segments were placed in 10% neutral-buffered formalin for fixation overnight at room temperature and subsequently paraffin-embedded.

Analysis of CF microbiome

The relative abundance of the major phyla present in the CF mice was determined using 16S rRNA sequencing and QIIME software. Intestinal contents from the distal small intestine were collected into sterile microcentrifuge tubes after laparotomy and isolation of the segment of interest. Samples were collected from three wild-type C57Bl/6 CF mice and sequenced.

Hematoxylin and eosin measurements

Histologic sections were created for the ileal segments and H&E stained. Using brightfield microscopy (Leica, Wetzlar, Germany), images were obtained at magnification between ×200 and ×250. VH and CD were measured using ImageJ software, with at least 20 measurements for each parameter made per group. Villi were chosen for measurement when intact from crypt-villus junction to crypt-villus junction and central lacteal present. Crypts were measured when intact from crypt-villus junction to crypt-villus junction and at least partial visualization of adjacent villi present. The investigator performing measurements was not aware of sample identity until after measurements were completed and recorded.

Immunohistochemisty

Crypt proliferation index (CPI) was calculated using Ki67 staining in the crypts, and apoptosis was assessed using the TUNEL assay by standard immunohistochemical protocols and chromogenic detection. CPI values were calculated as number of positive Ki67 cells per crypt and converted to a percentage. TUNEL values were calculated as number of positive TUNEL cells per villus and converted to a percentage. The investigator performing measurements was not aware of sample identity until after measurements were completed and recorded.

Statistical analysis

Statistical analysis was carried out using the Prism software (GraphPad, San Diego, CA). Groups were compared using Student's *t*-test with significance assumed when $P \leq 0.05$.

Results

Weight

Animals were weighed before sacrifice, and mean weights were as follows: CF, 24.3 ± 0.6 g; *A muciniphila*, 23.0 ± 0.4 g; *B eggerthii*, 22.8 ± 0.5 g; *C symbiosum*, 22.8 ± 0.8 g; and *E coli*, 22.3 ± 0.5 g.

Mucosal growth parameters

VH (mean ± SEM) for all groups were as follows: CF, 206 ± 3 μm; *A muciniphila*, 316 ± 7 μm; *B eggerthii*, 245 ± 4 μm; *C symbiosum*, 309 ± 8 μm; and *E coli*, 279 ± 7 μm. CD mean ± SEM for all groups were as follows: CF, 57 ± 1 μm; *A muciniphila*, 68 ± 2 μm; *B eggerthii*, 69 ± 2 μm; *C symbiosum*, 79 ± 2 μm; and *E coli*, 77 ± 1 μm. All measurements of VH and CD for gnotobiotic